EpCAM–Independent ApoStream™ Technology Isolates Circulating Tumor Cells from Blood of Patients with Various Types of Cancer

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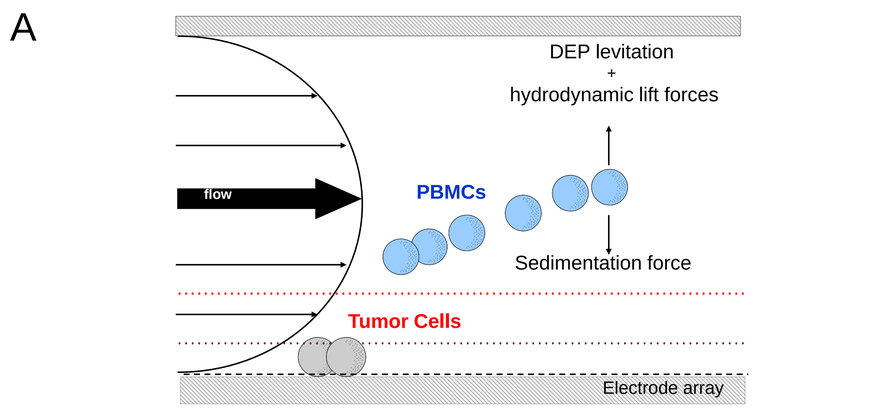
Abstract

Background: Circulating tumor cells (CTCs) are used clinically as biomarkers for monitoring metastatic disease progression. However, the use of EpCAM-based enrichment platforms limits the type of tumor cells that can be recovered, which subsequently limits the use of CTCs as surrogates for the classical tumor biopsy. Therefore an EpCAM-independent enrichment platform is critically needed. **Specific Aims:** To demonstrate the performance of a novel antibody-independent dielectrophoretic field-flow fractionation based CTC isolation technology ApoStream™ in both a spiked cell model and cancer patient blood. **Experimental Procedures:** We used ApoStream™, a novel antibody-independent technology, to enrich CTCs from the blood of prostate and breast cancer patients (high EpCAM expression), non-small cell lung cancer patients (NSCLC, low EpCAM expression) and melanoma patients (no EpCAM expression). Cells isolated from ApoStream™ were stained for cytokeratin (CK), CD45, and DAPI; melanoma CTCs with the tumor marker S100, CD45 and DAPI. Imaging and CTC enumeration were done using laser scanning cytometry (LSC). CTC morphology in lung cancer specimens was confirmed with H&E staining. **Results:** High CTC recovery from cancer patient blood samples was achieved with counts ranging from 0 – 2104 (lung, n=33), 0 – 3490 (prostate, n=15), 176 – 968 (breast, n=3), and 4 – 3120 (melanoma, n=11) CTCs per 7.5 mL blood. Positive CTC counts were obtained in 90% of NSCLC samples, 93% of prostate cancer samples, and 100% of both breast cancer and melanoma specimens. There were no false-positive CTCs from normal donor blood controls, demonstrating ApoStream™'s specificity. EpCAM-negative CTCs were also recovered in all tested breast cancer patient specimens, confirming that ApoStream™ isolates cancer cells independent of their EpCAM status.

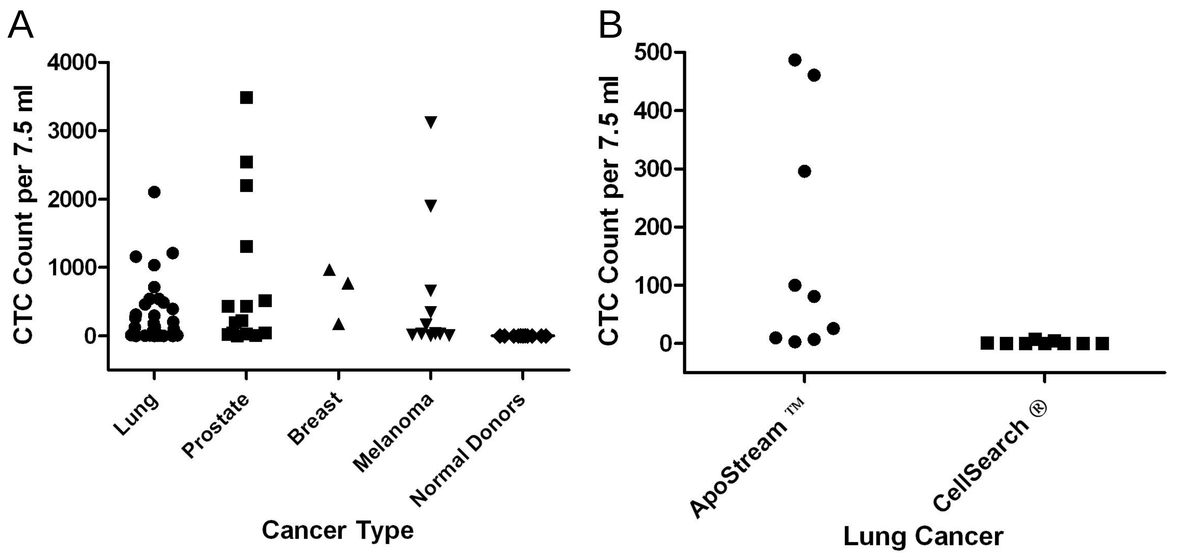
Conclusions: Our data demonstrate that the ApoStream™ platform recovers large numbers of CTCs from the blood of patients with metastatic NSCLC, prostate, breast cancer and melanoma. Thus, the ApoStream™ CTC isolation platform provides a new effective tool with broad applications in cancer biomarker discovery and personalization of cancer therapies.

ApoStream™ System



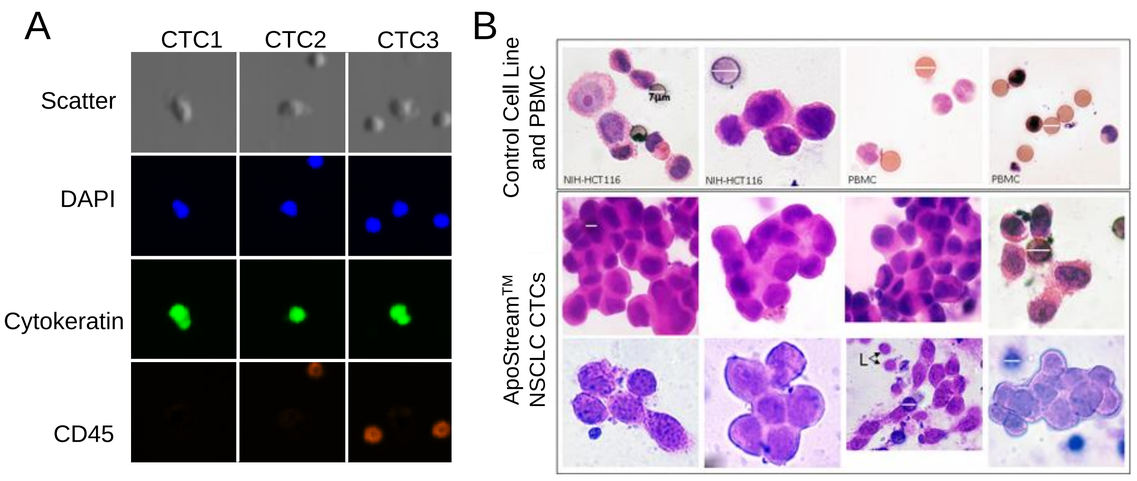
(A) A combination of forces dominated by dielectrophoretic forces, attract CTCs and repel normal cells from the chamber floor. CTC's are collected through a port located in the chamber floor while normal cells flow into a waste port. **(B)** Cross-over frequencies from over 20 different tumor cell types including breast, colon, ovarian, lung and melanoma cell lines and from peripheral blood mononuclear cells (PBMCs) were determined. The differences in cross-over frequencies between cancer and normal cells enable ApoStream™ to separate CTCs from normal cells.

High CTC Recovery Using ApoStream™



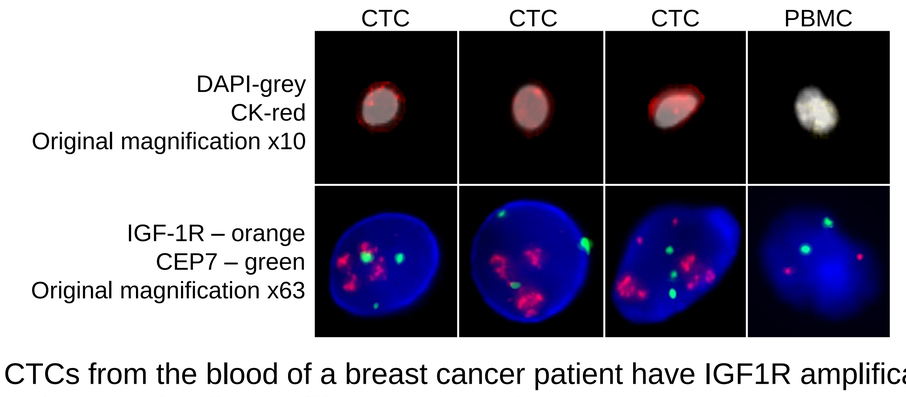
(A) ApoStream™ system was able to isolate high number of CTCs from lung, prostate, breast cancer, and melanoma patient blood, but not from the normal donor blood. **(B)** CTC isolation performance by our ApoStream™ system was far superior to the CellSearch® method for non-small cell lung cancer (NSCLC) patient blood.

LSC and H&E Analysis of CTCs



(A) CTCs isolated by ApoStream™ from blood of NSCLC patient were identified by immunofluorescent staining as $DAPI^{+}/CK^{+}/CD45^{-}$ cells. **(B)** CTC clusters were isolated from blood of lung cancer patients, as visualized by H&E staining.

FISH Analysis



CTCs from the blood of a breast cancer patient have IGF1R amplification. CTCs were isolated by ApoStream™ device, identified by immunophenotyping, and then examined by FISH.

Conclusions

- ApoStream™ is a high throughput CTC isolation system that permits antibody-independent enrichment of CTCs from various types of cancers with high recovery. ApoStream™ technology isolates higher number of CTCs from patients with NSCLC than the CellSearch® method, evidently due to capturing EpCAM-negative cancer cells.
- CTC capture with ApoStream™ allows downstream CTC characterization such as protein expression, gene expression, mutation analysis and FISH.
- ApoStream™ can serve as a new effective tool with broad applications in cancer biomarker discovery and implementation of personalized cancer therapy.

Acknowledgments

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